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METHOD FOR PRODUCING WATER-SOLUBLE SOY ISOFLAVONES
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[Claims] /2*

[Claim 1] A method for producing water-soluble soy isoflavones, said method comprising mixing a crude extract obtained from a soybean raw material with a cyclodextrin in an aqueous solution and subsequently eliminating insolubles.

[Claim 2] The production method stated in Claim 1, in which the pH of the aqueous solution at the time of mixing said crude extract and cyclodextrin is from 2 to 8.

[Claim 3] The production method stated in Claim 1, in which the temperature of the aqueous solution at the time of mixing said crude extract and cyclodextrin is from 5 °C to 40 °C.

[Claim 4] The production method stated in Claim 1, in which the aqueous solution at the time of mixing said crude extract and cyclodextrin does not contain any organic solvent.

[Detailed Description of the Invention]

[0001] [Industrial Field of the Invention]

The present invention pertains to a production method for obtaining water-soluble soy isoflavones that are effective for, for example, prevention of osteoporosis.

[0002] [Prior Art]

Isoflavone derivatives are known to be effective for adult diseases, such as osteoporosis, hyperlipemia, etc. Accordingly, consumption of soybeans that are rich in isoflavone derivatives is

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* Number in the margin indicates pagination in the foreign text.

recommended, and various methods for extracting them have been proposed.

[0003] As a method for producing soy isoflavones in large quantities, there is a method proposed in JP-A-S62-126186 that brings a soybean extract solution in contact with a synthetic resin and subsequently elutes isoflavones. The extract obtained by this method, however, has problems in that it does not dissolve well in an aqueous solution and that it is unstable in an aqueous solution. This extract not only forms precipitates in an aqueous solution in the acidic range, but, when its concentration is high, also forms precipitates over time even in an alkaline solution, in which the solubility of isoflavones is supposed to be higher.

[0004] As a method for blending flavonoids, such as isoflavones, etc., stably at a high concentration in an aqueous solution is known a method of inclusion with a cyclodextrin.

[0005] For example, in JP-A-H06-088993 is disclosed a method that prepares an inclusion complex of 7-isopropoxy-isoflavone with a cyclodextrin so as to increase its solubility. Similarly, in JP-A-S59-137499 is disclosed the preparation of a rutin inclusion compound with a cyclodextrin. These methods, however, use refined products having high purity and consequently produce high-cost products, and there is no mention of production methods that use soybean extracts.

[0006] In JP-A-S63-317059 is disclosed a cooked soybean production method, according to which, in the process of producing

heat-sterilized cooked soybeans, β-cyclodextrin or γ-cyclodextrin is added so as to prevent isoflavones from separating out during storage. However, the stability of an aqueous solution containing soy isoflavones at a high concentration is unknown.

[0007] Isoflavone derivatives are effective for the prevention and treatment of various diseases, but, in order to obtain their preventive effects against, for example, osteoporosis, approximately 50 mg/day intake is necessary. However, daidzin, for example, has an extremely low solubility of 0.0033 mg/mL in distilled water (25 °C), and, to take it 50 mg/day, which is its effective dosage, in the form of a beverage, it must be taken in too large a quantity to be feasible. Therefore, its solubility must be increased.

[0008] In addition, by making it water-soluble to a certain degree, its intestinal absorption can be increased.

[0009] [Problems that the Invention Intends to Solve]

The present invention intends to provide a method for producing water-soluble soy isoflavones that can dissolve in an aqueous solution at a high concentration and that have long-term stability.

[0010] [Means of Solving the Problems]

The present inventors researched extensively to solve the aforesaid problems and, as a result, learned a surprising fact that, by mixing a crude soybean extract with a cyclodextrin in an aqueous solution and by eliminating insolubles, water-soluble soy isoflavones that are highly water-soluble and that exhibit good long-term

stability in a dissolved state can be obtained. Based on this finding, the present invention was achieved.

[0011] The gist of the present invention is that a crude soybean extract obtained by, for example, solvent extraction, etc., is resuspended in an aqueous solution, into which are also mixed various types of cyclodextrins. Thereafter, insolubles are eliminated by centrifugation, filtration, etc. The obtained transparent solution that contains water-soluble soy isoflavones may be used as is or may be used after it is condensed or solidified. This production method requires very low production cost and can be implemented easily.

[0012] [Mode of Implementing the Invention]

According to the production method of the present invention, as the raw material soybeans are used whole soybeans, dehulled soybeans, defatted hypocotyls, soybean hypocotyls, soybean meal, or stock or whey generated in the production process of tofu or soy protein isolates, of which soybean hypocotyls, etc., which have a high isoflavone content, are preferably used.

[0013] The method for obtaining a crude soybean extract, which is the raw material used in the production method of the present invention, from the aforesaid raw material soybeans is not specifically limited, and the following process may be employed. In the case of using, as the raw material, a solid-form raw material, such as whole soybeans, dehulled soybeans, defatted hypocotyls, soybean hypocotyls, soybean meal, etc., they are first chopped and

pulverized with a grinder mill and then mixed with an extracting solvent, thereby preparing a crude soybean extract solution. As the extracting solvent, water or an organic solvent, such as alcohol (for example, methanol, ethanol, isopropanol, etc.) and ether (for example, acetone, etc.), is used. Preferably, a hydroalcoholic solution, especially an aqueous solution of 70 to 90 % alcohol, better yet, of approximately 80 % alcohol, is used. The extraction can be effected by immersion at room temperature, but it is preferable to perform reflux extraction at 70 °C or thereabouts because this causes malonyl, which /3 is an isoflavone glucoside, to degrade, thus improving the solubilization effect of a cyclodextrin. The obtained crude soybean extract solution is condensed or dried.

[0014] If the isoflavone content in the obtained crude soybean extract solution is low, it is further condensed using a synthetic resin. Crude extracts having high-concentration isoflavones can be obtained by the method disclosed in JP-A-S62-126186 or the method proposed by Kitagawa, et al., (Journal of the Japanese Society for Food Science and Technology, 33 (12), pp. 821-825, **1986**). As the synthetic resin, a porous styrene-divinyl benzene resin, such as Daiya Ion HP-20 (a product of Mitsubishi Chemical Industries), Amber Light XAD-2 and XAD-4 (products of Rohm and Haas Co.), and Dulite S-861 and S862 (products of Sumitomo Chemical Industries), is used, and it is packed in a column in a quantity of, for example, from 1/50 to 1/5 times the quantity of the solution, thereby refining the crude soybean

extract solution. As the eluent, a 30 to 80 % hydroalcoholic solution is used. However, since glucosides are more readily solubilized than aglycons with a cyclodextrin, it is preferable to use a 30 to 50 % hydroalcoholic solution so as to recover isoflavones mainly comprised of glucosides because this can yield isoflavones that are more stable in final product beverages. From the concentrate, the organic solvent is completely eliminated by heating and pressure reduction.

[0015] With this process, a crude soybean extract whose isoflavone content in the solid component is 20 to 90 %, usually 20 to 70 %, preferably 30 to 50 %, can be obtained. The other components are comprised of soybean saponins and soybean proteins.

[0016] The present invention pertains to a method for producing a soy isoflavone extract whose stability in an aqueous solution is increased from the crude soybean extract thus obtained by the following operation. That is, the crude soybean extract, together with various types of cyclodextrins, is suspended and mixed in an aqueous solution. Thereafter, insolubles are eliminated by centrifugation, filtration, etc., thereby obtaining transparent water-soluble soy isoflavones.

[0017] First of all, a crude soybean extract is suspended in water. Here, the concentration of soy isoflavones is set to from 0.1 to 100 mg/mL, preferably from 0.1 to 10 mg/mL. Incidentally, the quantity of soy isoflavones can be determined as follows. A specimen that has been diluted as appropriate is subjected to high-performance

liquid chromatography under the following conditions for quantitative analysis: column, silica gel reverse-phase ODS column; mobile phase, acetonitrile: water: acetic acid = from 15: 84.9: 0.1 to 35: 64.9: 0.1; flow rate, 1.0 mL/min.; column temperature, 35 °C; and detection method, UV at 260 nm. The sum of the daidzin and genistin quantities indicated in the chromatogram thus obtained is considered to be the soy isoflavone quantity.

[0018] As the cyclodextrin to be mixed, α-, β-, and γ-cyclodextrins, G2β-cyclodextrin, G1β-cyclodextrin, and mixtures of these can be used. Of these, β-cyclodextrin, G2β-cyclodextrin, and G1β-cyclodextrin are especially preferable. The concentration of cyclodextrin is in the range of from 2 to 200 mg/ml, preferably from 10 to 100 mg/mL, in said aqueous solution. It is not desirable to use cyclodextrin in an amount that exceeds the aforesaid range because it is not economical, and, in addition, the solubility of cyclodextrin proper becomes problematic, depending on the type of cyclodextrin.

[0019] The ratio of the soy isoflavone crude extract to cyclodextrin is from 1: 0.5 to 1: 50, preferably from 1: 5 to 1: 15, in terms of weight ratio.

[0020] The ratio of the soy isoflavones to cyclodextrin is preferably from 1: 2 to 1: 20 in terms of weight ratio. If the ratio is lower than 1: 2, soy saponins and soy proteins, which cause interactions between the components contained in the extract during a storage period, are also dissolved and, consequently, form

precipitates, thus resulting in insufficient stability. If it is higher than 1: 20, the weight of cyclodextrin becomes unnecessarily large when the resulting product is formed into powder; thus, this ratio is not desirable from the standpoint of the product form as well as cost efficiency. The pH of the aqueous solution in the mixing process should be adjusted to from 2 to 8, preferably from 3 to 6. The acid to be used for adjusting the pH of said aqueous solution may be any inorganic or organic acid as long as it can be applicable to food, and some examples include hydrochloric acid, citric acid, malic acid, acetic acid, phosphoric acid, ascorbic acid, etc. If the pH exceeds 8, although the resulting product stays dissolved temporarily during the present process, it causes other components, such as soy saponins, soy proteins, etc., in soybeans to be dissolved in large quantities, and, consequently, precipitates will be formed during storage when the resulting product is mixed into liquid beverages, such as acidic beverages, etc. A pH below 2 gives rise to a concern for the deterioration of the water-soluble soy isoflavones proper.

[0021] The mixing is conducted at a temperature in the range of from 5 °C to 40 °C, preferably from 15 °C to 25 °C. If the temperature exceeds 40 °C, although the resulting product stays dissolved temporarily during the present process, the components that cause precipitates after cooling are mixed into the resulting product. Furthermore, this aqueous solution should not contain any organic solvent, such as ethanol, etc. The reason for this is that, if the

present process is carried out using an aqueous solution containing an organic solvent, soy isoflavones that temporarily exhibit good solubility can be obtained, but precipitates will be formed thereafter over time. Accordingly, it is necessary to eliminate organic solvents beforehand even from the raw material crude soybean extract.

[0022] With the aforesaid production process, intrinsically water-insoluble soy isoflavones that are obtained by condensing a soybean extract are, surprisingly, made soluble, and, moreover, the quantities of soy saponins, soy proteins, etc., are reduced to from 1/2 to 1/20 of the quantities before the process, thereby improving the product stability in an aqueous solution. By further subjecting a suspension obtained by mixing the aforesaid crude soybean extract and cyclodextrin to such a process as centrifugation, filtration, etc., a transparent soy isoflavone aqueous solution having a high soy isoflavone content can be obtained.

[0023] The centrifugation is conducted preferably at 1000 to 4000 rpm for 20 to 120 minutes.

[0024] Said mixture suspension is filtered when necessary. The filtration is conducted with a filter of approximately 0.1 to 5 μm acetic acid cellulose, etc.

[0025] The filtrate thus obtained, which is a soy isoflavone aqueous solution, has a high isoflavone concentration of approximately 0.1 to 4.5 mg/mL, and, compared with the solubility of one kind of /4

soy isoflavone daidzin, which is 0.0033 mg/mL, the concentration is increased approximately 1000 times.

[0026] This filtrate may be blended in beverages as is or may be concentrated and dried for the purpose of preservation until the blending. In terms of dry weight, the soy isoflavones comprise 0.1 to 90, preferably 0.5 to 50, better yet, 1 to 20, % by weight, of the product, and the remainder is in effect mostly cyclodextrin.

[0027] The water-soluble isoflavones thus obtained may be prepared into a beverage and served. In this case, 0.1 to 4.5 mg/mL of soy isoflavones can be incorporated into the beverage. They may also be provided in a solid form, such as tablets, granules, etc.

[0028] [Working examples]

The following explains the present invention in further detail, referring to experiment examples and working examples, but it should be borne in mind that the present invention is not limited to or restricted by these.

[0029] Experiment Example 1

Study of the cyclodextrin addition conditions

In each of several test tubes, 50 mg of a crude soybean extract (20 mg in terms of isoflavone weight) and 100 mg of β-cyclodextrin were suspended with 5 mL of various types of solvents at varying pH and temperature. Here, the pH was adjusted with citric acid or an aqueous solution of sodium hydroxide. Thereafter, each test tube was shaken for 10 minutes, and the content was centrifuged (2000 rpm, 60

min.) and filtered with a 0.45 μm filter, after which the filtrate was vacuum-dried, thereby obtaining a dried product. Considering its application to acidic beverages, the obtained powder in a quantity of 50 mg in terms of soy isoflavones was redissolved in 50 mL of water, and the pH of the solution was adjusted to 3.8 with citric acid. With this solution, its stability at 40 °C for 1 month was tested. The test results are shown in Table 1.

[0030] [Table 1]

STUDY OF CYCLODEXTRIN ADDITION CONDITIONS

Solvent	pH	Temperature	Stability Test
100% ethanol	6	20	X
40% ethanol	6	20	X
20% ethanol	6	20	X
Water	12	20	X
Water	10	20	X
Water	9	20	X
Water	8	20	O
Water	7	20	O
Water	6	20	O
Water	4	20	O
Water	2	20	O
Water	9	80	X
Water	9	60	X
Water	9	40	X
Water	9	5	X
Water	6	80	X
Water	6	60	X
Water	6	40	O
Water	6	5	O

X: Precipitates were formed, O: No precipitate was observed.

[0031] (1) Effect of pH

When the present process was conducted with aqueous solutions having a pH of 9 or higher, the filtrate dissolved completely temporarily, but precipitates were formed in the stability test under any conditions. However, it was learned that, when the present process was carried out at a pH of 8 or less, saponins, soy proteins, etc., which interact with the solubilized isoflavones during storage and cause precipitation, could be isolated easily.

(2) Effect of Solvent

When the present process was carried out in an alcohol solution, the dried powder of the filtrate containing soy isoflavones dissolved completely temporarily. However, it precipitated after a month in the stability test.

(3) Effect of Temperature

When the present process was carried out under a heated condition of 60 °C or thereabouts, as in the case of processing in an alcohol solution, the product dissolved completely temporarily but precipitated in the stability test under any conditions. /5

[[0032] Experiment Example 2

Study of the cyclodextrin addition quantity

In a test tube containing 5 mL of an aqueous solution, 50 mg of a crude soybean extract (20 mg in terms of isoflavone weight) and 1, 10, 50, 100, 150, 200, or 300 mg of a different kind of cyclodextrin were suspended under the cyclodextrin-addition conditions obtained as a

result of Experiment Example 1 (pH < 9, 20 °C), and the test tube was shaken for 10 minutes. Thereafter, the content was centrifuged (2000 rpm, 60 min.) and filtered with a 0.45 μm filter, after which the filtrate was vacuum-dried, thereby obtaining a dried product. The obtained powder in a quantity of 50 mg in terms of soy isoflavones was redissolved in 50 mL of water, and the pH of the solution was adjusted to 3.8 with citric acid. With this solution, its stability at 40 °C for 1 month was tested. The test results are shown in Table 2.

[0033] [Table 2]

STUDY OF CYCLODEXTRIN ADDITION QUANTITY

Soy Isoflavone Crude Extract	Cyclodextrin Addition Quantity (mg/mL)	Stability Test	
		β-cyclodextrin	G2β-cyclodextrin
Glucosides (including malonyl)	0.2	X	X
	2	X	X
	10	X	O
	20	O	O
	30	O	O
	40	O	O
	60	O	O
Glucosides (excluding malonyl)	0.2	X	X
	2	O	X
	10	O	X
	20	O	O
	30	O	O
	40	O	O
	60	O	O

X: Precipitates were formed, O: No precipitate was observed.

[0034] (1) Effect of the soy isoflavone constitution

Solubility was better when glucosides were used compared with the case of using aglycons. Glucosides from which malonyl had been eliminated exhibited the best solubility.

(2) Effect of the addition quantity of cyclodextrin

When the addition quantity of cyclodextrin was 100 mg or less, the filtrate dissolved completely temporarily, but it precipitated in the stability test. On the other hand, when the addition quantity of cyclodextrin was 100 mg or more, the stability was good under any condition.

[0035] Effect of cyclodextrin type

Compared with unbranched β-CD, the use of branched cyclodextrin G2β-CD and cyclodextrin containing G2β-CD resulted in higher solubility values.

[0035] Working Example 1

As the soybean raw material, 50 kg of soybean hypocotyl was pulverized and subjected to reflux extraction with 250 L of an 80 % alcohol aqueous solution at 70 °C, and the crude soybean extract solution was condensed under vacuum to approximately 50 L. This solution was adsorbed to Daiya Ion HP-20 (a product of Mitsubishi Chemical Industries), thereby refining the crude soybean extract solution. A 40 % alcohol solution was used as the eluent here. The organic solvent was completely eliminated from the concentrate by heating and pressure reduction. With this process was obtained 950 g of a crude soybean extract whose soy isoflavone content in the solid was 42 %. This crude soybean extract was used as the starting material in the production method of the present invention. First, said crude soybean extract was suspended in 95 L of water so as to set its

concentration to 10 mg/mL in terms of soy isoflavones. The pH was adjusted to pH 5 by adding malic acid powder gradually in small quantities. G2β-cyclodextrin was added to the above suspension so as to set its concentration to 50 mg/mL. They were mixed and stirred for about 2 hours at 20 °C. Thereafter, the mixture was centrifuged with a centrifugal separator at 2500 rpm for 60 minutes. It was further filtered with a filter of 1 μm or thereabouts. The obtained transparent solution was heated and condensed, thereby obtaining 600 g water-soluble soy isoflavones. The soy isoflavone content of this product was 19 % by weight.

[0036] Working Example 2

As the soybean raw material, 50 kg of defatted hypocotyl was pulverized and subjected to reflux extraction with 250 L of an 80 % alcohol aqueous solution at 70 °C, and the crude soybean extract solution was condensed under vacuum to approximately 40 L. This solution was adsorbed to Daiya Ion HP-20 (a product of Mitsubishi Chemical Industries), thereby refining the crude soybean extract /6 solution. A 40 % alcohol solution was used as the eluent here. The organic solvent was completely eliminated from the concentrate by heating and pressure reduction. With this process was obtained 900 g of a crude soybean extract whose soy isoflavone content in the solid was 35 %. This crude soybean extract was used as the starting substance in the following process. First, said crude soybean extract was suspended in 110 L of water so as to set its concentration to 8

mg/mL in terms of soy isoflavones. The pH was adjusted to pH 5 by adding malic acid powder gradually in small quantities. Isoelite P, a product of Nikken Chemical Co., was added to the above suspension so as to set its concentration to 100 mg/mL. They were mixed and stirred for about 2 hours at 25 °C. Thereafter, the mixture was centrifuged with a centrifugal separator at 2500 rpm for 60 minutes. It was further filtered with a filter of 1 μm or thereabouts. The obtained transparent solution was heated and condensed, thereby obtaining 550 g water-soluble soy isoflavones. The soy isoflavone content of this product was 15 % by weight.

[0037] [Effects of the Invention]

The present invention provides a method for producing water-soluble soy isoflavones that dissolve in an aqueous solution at a high concentration and exhibit good long-term stability.